

Michigan's Experience of Screening for Severe Combined Immune Deficiency Syndrome (SCID): Summary of First Year.

H. WOOD¹, C. Burns¹, K. Andruszewski¹, M. Kleyn¹, E. Secord², M. Ruehle², M. Tierce², K. Cavanagh¹ Michigan Department of Community Health¹, Lansing, MI; ²Children's Hospital of Michigan, Detroit, MI



Abstract

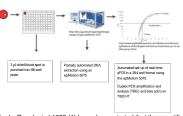
Objectives: To assess the program outcomes of Michigan (MI) newborn screening from the first year using a duplex quantitative real time PCR (qPCR) assay for T-cell receptor excision circles (TREC) and beta actin for SCID and other primary immune deficiency disorders.

Methodology: All MI newborns were screened beginning 10/1/2011. Prior to qPCR analysis, an automated DNA extraction with a 3 mm punch from a dried blood spot (DBS) was performed for sample preparation. Follow-up of very low TREC values, "strong positive" results, was coordinated by one center with referrals to three specialty centers. Three algorithm changes were implemented as more experience with this laboratory-developed method was obtained to reduce the number of referrals.

Results: Over 111,000 infants had initial screens tested for SCID; 0.34% had "abnormal" (inconclusive, borderline, or strong positive) results. Approximately 70% of the infants with abnormal results were in Neonatal ICU. A majority of these "abnormal" samples resolved by either a normal repeat TREC test on a new DBS or flow cytometry testing. There were no classic cases of SCID discovered in this period. There were 9 cases of Syndromes with T cell impairment (e.g., DiGeorge/22q11.2 deletion syndrome; CHARGE with DiGeorge, T=B+ NK+); 11 cases of secondary T cell lymphopenia other than preterm alone (e.g., cardiac surgery/thymectomy; malignancy); and 3 cases of idopathio/transient lymphopenia. Conclusions: A summary of the 1st year of SCID screening in MI will be reported. Use of a borderline positive category with cutoffs based on birth weight reduced the number of lites positive category with cutoffs based on birth weight reduced the number of lates positive cases are in progress and will be reported. Clinical follow-up of these secondary immunodeficiencies will be summarized. "Partially funded by Grant 101EH000398 from CDC/NCEH.

Methods

Figure 1: Overall Summary of MI method for TREC analysis



Methods: Douek et al 1998 (1) have demonstrated that the quantification of T cell receptor excision circles (TRECs) using quantitative real time PCR (qPCR) will estimate naive T cells that recently emigrated from the thymus. Several laboratories have adapted this method for TREC analysis which made it feasible to include SCID in newborn screening (2, 3). Wood et al (4) have modified this further to include a single reagent extraction partial automation of the DNA extraction from the dried blood spot (DBS) and TREC, B-Actin duplex qPCR. TREC and β -Actin plasmids were obtained from Dr. John Routes and calibrators were prepared in house. An independent validation of these calibrator assignments were verified by digital PCR performed at CDC (Drs Voglt, Lee and Taylor, personal communication).

MI Algorithm

Figure 2: Flow diagram of current MI algorithm



Figure 2: Flow diagram of current MI algorithm: Five algorithm changes were implemented as more experience with this laboratorydeveloped method was obtained to reduce the number of referrals from the NICU. Initial cutoffs were very conservative to avoid false negatives and to get a better understanding of the secondary T cell lymphopenia cases detected. Algorithm changes are available in a handout, Follow-up of borderline positive and inconclusive results is coordinated by NBS epidemiology group. Follow-up of strong positive results is coordinated by one center with referrals to three specialty centers as indicated in the flow

Results

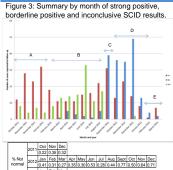


Figure 3: Summary by month of SCID results, Letters A-E signify different TREC, B-actin screening algorithms used as more experience was gained. Strong positive indicated direct referral, borderline and inconclusive (amplification failure) results indicated a repeat DBS. Over 162,000 infants had initial screens tested for SCID using the dunley TREC, 6 Actin. described. Overall, 0.4% had "abnormal" (inconclusive=inc, borderline= B+ or strong positive= S+) results although the abnormal percent has decreased to 0.1% in recent

"Providing Quality Laboratory Science for Healthier People and Communities"

diagram.

Results

Table 1: Summary of Nine True Positive Cases from MI

Zamaja	Elithonight (grams)	Ossistional age	Collection are based	TREC copiestic	TRUC CT	ALC from CRC-4R	CO3+ T cells.	CDI Toella losbidi	CDS T with			CDG native T cells (sells, id., and % of total CDS T sells)	setts (setted)			Controval result
	tan:	30	26	2	38.86	1000	138	328	100	7	405	D.4%, 188	87.7%; 211	30%; 110	1.6%; 4	Arlama helensiyyasa mulalisn causing hypomorphic SCID
2	1820	23	26	0	38.38	1300	44	39	0	624	338	88%	NA	70%	NA.	Helenoggout mulation in the RSSP gene consistent with degrates of RSSP associated SCD
3	3335			7	37.09	No.	165	760	760	п	400	275	738	101	75	Contined Invarie Delateray
4	1619	в	3	٠	+60.00	-		2	٠	863	218	No.	NA.	NA.	NA.	OHREE syndrome with Difference 22s/11.2 deletion
٠	3340	39	29	۰	+65.00	1400	140	**	a	ans.	140	29%	8%	79%	28%	Differency with 22q11 deletion
4	2030	28	3		373	1300		300	140	400	10	NA.	N/A	NA	NA.	"seuere" Plantal DiCleorge without 23q11 deterior
7	2790	34	30	14	36.5	-	204	100	27	10	266	NA.	NA.	NA	NA.	stophelosom
	3627	и	29	*	340	400	3072	1360	1006	1064	628	675,200	89%, 2002	675, 2864	36% 1720	Partial DiCleonge with 20q11 detection
	2006		28	7	37.84	-	1309	1031	378	**	40	66,7%,769	17.6% 388	16.0% 107	13%4	lymphopenia

Table 2: Overall Summary of MI Results October 1, 2011 through March 20, 2013

Date NBS- SCID	Total # of infants screened (indicate start and end date of data	analytical criteria for state for True positive) : includes pre- term alone	referred for flow cytometry: includes pre-term alone	cases for repeat NBS: includes pre-term alone	Total # Pre-term alone infants identified by	Total # of non- pre-term alone infants identifie d by NBS-	f/u = 1; Total # pre- term referred for f/u = 2 (e.g. 1= AA; 2 =	Total # SCID cases (1, 2,	Total # non- SCID/T cell lymphopeni a cases (excluding SCID cases) [4, 5, 6, 7, 8, 9]
10/1/2011	162528	434	234	200	214	178	1 = 86	3	35
							2 = 148	_	
	Date NBS- SCID nitiated 10/1/2011	Total # of infants screened Oate (indicate start NBS- and end date OCID of data initiated provided)	Total # of riteria for state for sta	Total 8 of Tot	Total # of criteria for ferende for season and total # of criteria for ferende for season and total # of criteria for flow ferende for season and end date includes processed and end date includes processed for form of criteria for form of criteria form of crite	Total # of minatas and minatas	Total 8 of Indians I	Total 8 of interest of the control	Total 8 of infrares Total 9 of infrares

Tables 1 and 2: There were 434 infants of 162.528 (0.26%) that required some level of follow up (Table 2), 234 of 434 (54%) had flow cytometry with the remainder having repeat DBS. 214 (49%) of the infants with decreased TREC values were preterm defined as <37 weeks although more recently these preterm cases have decreased 6-fold because of program initiatives. There were 3 classic cases of SCID (HRSA region 4 categories 1-3) observed in this period (Table 1). Case 1 was Artemis heterozygous mutation causing hypomorphic SCID; case 2 was Cartilage Hair Hypoplasia Syndrome with compound heterozygote mutations in RMRP gene; case 3 was a combined immune deficiency. In addition, there were 35 cases of non-SCID T cell lymphopenia comprised of 14 cases of Syndromes with T cell impairment (e.g., DiGeorge/22q11.2 deletion syndrome; CHARGE with DiGeorge, T-B+ NK+); 15 cases of secondary T cell lymphopenia other than preterm alone (e.g., cardiac surgery/thymectomy; malignancy); and 6 cases of idiopathic/transient lymphopenia/pending

Case Summary

SCID Case #2 Timeline

Born by C-section at 33 weeks gestation.

Day 1- Newborn Screen: Strong positive for SCID, TREC undetectable.

Day 3- Chromosome microarray and karvotype: normal

Day 7- Chromosome microarray and karyotype: normal Day 7- Flow cytometry showed low T cells (CD3+ of 65 (5%), CD4+ of 39 (3%), and CD8+ of 0), normal B cell and NK cell counts. Concern for T-

(3%), and cober of 0, nominal a centarion NA centarions. Concern for 1-/8+/NK+ SCID vs. DiGeorge syndrome.

Days 10 to 15-Transferred to Children's Hospital of Michigan for further

evaluation and care. Initial evaluation by genetics, immunology, and bone marrow transplant teams.

Patient was discharged home with breast milk and isolation precautions. **Day 30-** Bactrim prophylaxis, monthly IV immunoglobulin, Azithromycin

started.

Day 60- HLA typing showed no identical matched related or matched

unrelated donors.

Duke University (Dr Buckley) contacted for further evaluation and

consideration for T-cell depleted haploidentical transplant.
Genetic testing showed compound heterozygote for mutations in RMRP gene
(r.153 a-2 and r.231 c-2)).

The diagnosis of Cartilage Hair Hypoplasia Syndrome and associated combined immunodeficiency requiring hematopoietic stem cell transplant was made.

Day 90 – Matched unrelated donor found and preparing for reduced intensity hematopoietic stem cell transplant

Conclusions

- 1. A summary of the Michigan experience of SCID screening is provided
- 2. The incidence of SCID is 1 in 54,176 and the incidence of significant T cell lymphopenia is 1 in 4,392.
- Several algorithm changes were used to reduce the number of false positive and inconclusive cases.
- Newborn Screening Laboratory and Follow-up working with specialists and transplant surgeons at three Michigan medical centers are providing comprehensive services.

References

 Douek, DC et al, Nature 1998; 396, 690-695; 2 Chan K, and Puck JM. J Jamunol. 2009; 1216; (2): 391-8. 3. Baker MW et al J. Allergy Clin Immunol. 2009; 124(3): 522-527. 4. Wood et al 2013, "An improved method for DNA extraction method from Dried Blood Spots for T receptor excision circle (TREC) analysis and other Newborn Screening assays" Newborn Screening & Genetic Testing Symposium, May 5-10, Atlanta, GA

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Staff and Management of Michigan Newborn Screening Laboratory